

What is claimed is:

1. A method for increasing viral vector titer in a vector packaging cell comprising:

5 contacting a vector packaging cell with a viral vector, said

viral vector comprising a viral packaging signal sequence and a nucleotide sequence, the presence of which is desired in a host cell, said packaging cell capable of expressing structural viral components so that said viral vector may be assembled to form an infectious viral particle; and

10 inhibiting the presence of 5' methylated helper virus in said cell.

15 2. The method of claim 1 wherein said step of inhibiting methylation comprises:
positively selecting helper virus which is functional.

20 3. The method of claim 2 wherein said selection is by antibiotic resistance.

25 4. The method of claim 3 wherein said antibiotic resistance selection is accomplished via ligation of an internal ribosome entry site with a selection marker so that drug selection ensures promoter function in said helper virus.

30 5. The method of claim 1 wherein said viral titer achieves levels of 1.5×10^7 cfu/ml in the presence of antibiotic resistant selection.

35 6. The method of claim 1 wherein said helper virus comprises at least one viral production gene operably linked to viral promoter sequence which is capable of being methylated.

7. The method of claim 6 wherein said viral promoter comprises a long term repeat.

8. The method of claim 7 wherein said viral promoter sequence is the LTR of retrovirus.

5 9. The method of claim 1 wherein said step of decreasing inactive helper virus comprises the step of:
inhibiting methylation of helper virus.

10 Sub.D 10 10. The method of claim 9 wherein said inhibiting of methylation is accomplished by: a step selected from the group consisting of:
treating of vector producer cells with 5-AZA-C.

15 11. The method of claim 9 wherein said inhibiting of methylation is accomplished by: a step selected from the group consisting of:
insertion of a demethylation fragment of murine thy-1 in front of the 5' long terminal repeat.

20 12. The method of claim 9 wherein said inhibiting of methylation is accomplished by: a step selected from the group consisting of:
immune response selection.

25 13. The method of claim 9 wherein said inhibiting of methylation is accomplished by: a step selected from the group consisting of:
design of synthetic viral promoters to omit methylation sites.

30 14. The method of claim 9 wherein said inhibiting of methylation is accomplished by: a step selected from the group consisting of:
introducing a drug which inhibit methylation

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15. The method of claim 9 wherein said inhibiting of methylation is accomplished by: a step selected from the group consisting of:
antisense inhibition of the human methylase gene.

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16. A helper virus nucleotide sequence comprising:
a packaging deficient nucleotide sequence which encodes one or more structural viral components necessary for assembling a viral capsid, a viral promoter sequence capable of becoming methylated and a marker selection gene placed so that active helper virus may be positively selected.

17. The nucleotide sequence of claim 16 wherein said viral promoter sequence comprises:
a long terminal repeat promoter sequence which has been modified to inhibit methylation.

18. The helper virus nucleotide sequence of claim 16 wherein said sequence includes the methylation fragment of murine thy-1 in front of the 5' long terminal repeat site.

19. The helper virus nucleotide sequence of claim 16 wherein said sequence includes an internal ribosome entry site with a selection marker downstream of a viral component encoding sequence so that selection ensures promoter function.

20. The helper virus nucleotide sequence of claim 16 wherein said internal ribosome entry site is a picornavirus internal ribosome entry site.

21. The helper virus nucleotide sequence of claim 16 wherein said marker selection gene is an antibiotic resistance marker.

22. The helper virus nucleotide sequence of claim 16 wherein said helper virus is pAM3-IRES-Zeo.

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23. A vector packaging cell, said cell comprising a helper virus nucleotide sequence according to claim 16.

5 24. A vector producer cell comprising a helper virus nucleotide sequence according to claim 16 and a viral vector, said producer cell capable of assembling virions particles.

10 25. An infectious viral particle produced by the method of claim 1.

Substantive
26. A method for increasing viral titer produced by a vector packaging cell upon transfection with a viral vector comprising:

15 decreasing the amount of inactive helper virus present in said vector packaging cell by providing for the elimination of or prevention of methylated helper virus.

20 27. The method of claim 26 wherein said step of decreasing inactive helper virus comprises the step of: inhibiting methylation of helper virus.

25 28. The method of claim 26 wherein said step of decreasing inactive helper virus comprises the step of: removing from a population of vector packaging cells, helper virus with 5' long terminal repeat methylation.

30 29. The method of claim 28 further comprising the step of: removing cells with inactivated virus by positive selection.

35 30. The method of claim 29 wherein said removal step comprises: introducing an antibiotic to said cells so that cells with inactive helper virus are killed.

31. The method of claim 30 wherein said removal is accomplished by a helper virus with a picarnovirus internal

Substantive
ribosomal entry site sequence followed by an antibiotic resistance marker at the 3' end of the env sequence of said helper virus.

5 32. The method of claim 31 wherein said antibiotic resistance selection marker is Zeocin.

10 33. The method of claim 27 wherein said inhibiting of methylation is accomplished by a step selected from the group consisting of:
treating of vector producer cells with 5-AZA-C.

15 34. The method of claim 27 wherein said inhibiting of methylation is accomplished by a step selected from the group consisting of:
insertion of a demethylation fragment of murine thy-1 in front of the 5' long terminal repeat.

Substantive
20 35. The method of claim 27 wherein said inhibiting of methylation is accomplished by a step selected from the group consisting of:
ligation of an internation ribosome entry site with a selection marker so that drug selection would ensure promoter function.

25 36. The method of claim 27 wherein said inhibiting of methylation is accomplished by a step selected from the group consisting of:
immune response selection.

30 37. The method of claim 27 wherein said inhibiting of methylation is accomplished by a step selected from the group consisting of:
design of synthetic viral promoters to omit methylation
35 sites.

38. The method of claim 27 wherein said inhibiting of
methylation is accomplished by a step selected from the
group consisting of:
screening for and identification of other drugs which inhibit
methylation.

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39. The method of claim ~~27~~ wherein said inhibiting of
methylation is accomplished by a step selected from the
group consisting of:

10 an antisense inhibition of the human methylate gene.

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